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Cadmium Toxicity to Three Species of Estuarine Invertebrates

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CADMIUM TOXICITY TO THREE SPECIES OF ESTUARINE INVERTEBRATES

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ABSTRACT

Three species of estuarine invertebrates, Palaemonetes pugio (*grass shrimp*), Pagurus longicarpus (*hermit crab*) *and* Argopecten irradians (*bay scallop*), *were exposed to Cd in flowing seawater at concentrations of* 0·06, 0·12, 0·25, 0·5 *and* 1·0 *mg/litre. Incipient* LC_{50} *values of* 0·53 *and* 0·07 *mg/litre were estimated for bay scallop and hermit crab, respectively. The toxicity curve for grass shrimp had not stabilised, but the incipient* LC_{50} *value was estimated to fall within a range of* 0·2 *to* 0·3 *mg/litre. Short-term response, as measured by time to* 50% *mortality at the highest Cd concentration, was* 10, 21 *and* 23 *days for the bay scallop, hermit crab and grass shrimp, respectively. Scallop growth was inhibited at all exposure concentrations with a measured* 42*-day* EC_{50} *value of* 0·078 *mg/litre Cd. Byssal thread detachment precedes death in bay scallops. An* EC_{50} *value of* 0·54 *mg/litre Cd for byssal detachment was measured on day* 8 *of the bioassay before appreciable mortality. This compared favourably with the incipient* LC_{50} *value of* 0·53 *mg/litre Cd. Cadmium accumulation occurred at all concentrations in bay scallop and grass shrimp.*

INTRODUCTION

Many tons of cadmium are introduced annually to coastal marine environments; for example, via sludge dumping and municipal wastewater outfalls (Young, 1974; Mueller *et al.*, 1976; Pesch *et al.*, 1977). Elevated cadmium concentrations are found in sediments and waters of impacted areas, particularly along industrialised coastlines (Holmes *et al.*, 1974; Young *et al.*, 1975). Marine organisms exposed to

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elevated cadmium levels have higher tissue concentrations of cadmium than organisms from clean areas (Peden *et al.*, 1973; Ratkowsky *et al.*, 1974; Pesch *et al.*, 1977). In one instance elevated cadmium concentrations have been traced as far as 90 miles from a source (Butterworth *et al.*, 1972). Water quality criteria used to protect marine organisms from cadmium insults are based primarily on short-term static acute bioassays. Long-term continuous flow bioassay systems are thought to provide more sensitive responses and better approximations of 'real world' conditions (Ball, 1967). This study provides toxicological data obtained in flowing water systems using cadmium on three species of estuarine invertebrates, *Palaemonetes pugio* (grass shrimp), *Pagurus longicarpus* (hermit crab) and *Argopecten irradians* (bay scallop).

MATERIALS AND METHODS

Adult grass shrimp were collected from a tidal marsh in Narragansett Bay, Rhode Island, in October, 1972. The shrimp ranged from 2·0 to 3·3 cm in length and from 0·04 to 0·34 g wet weight. Adult hermit crabs were collected from Pt. Judith Pond, Narragansett, Rhode Island, a coastal RI salt pond, in November, 1972. The crabs ranged from 0·35 to 0·93 g wet weight. Bay scallops were obtained in March, 1973 as juveniles from a hatchery at Wachapreague, Virginia, maintained by the Virginia Institute of Marine Science. The scallops ranged in length of longest rib from 1·1 to 1·7 cm and from 0·51 to 0·73 g wet weight of soft parts. All test animals were maintained in flowing, unfiltered Narragansett Bay seawater at ambient conditions and acclimated in the laboratory for at least a month before treatment. No mortalities were observed during holding.

Flowing water proportional diluters, as described by Mount & Brungs (1967), were used. These diluters were constructed entirely of glass and calibrated to deliver one litre of seawater every two minutes to each of two 40-litre aquaria for each concentration. Five concentrations plus controls were used. The flow rates gave an approximate 99% volume replacement four times a day (Sprague, 1969). Each diluter was maintained and cleaned daily to ensure uninterrupted function.

Cadmium was administered from a stock solution (2·1 g Cd/litre) made up with reagent grade $CdCl_2 2{\cdot}5\ H_2O$ and deionised water. A Mariotte bottle was used to deliver the stock to the diluter. The diluter was adjusted to deliver nominal concentrations of 0·06, 0·12, 0·25, 0·50 and 1·0 mg/litre and controls. Seawater cadmium concentrations were determined twice weekly in samples from the aquaria using a Perkin-Elmer model 303 atomic absorption instrument (mention of a product name does not constitute endorsement) using methods described in the manual which accompanied the instrument (Perkin-Elmer Corporation Inc., 1966).

Each species was tested separately in aquaria arranged randomly with random distribution of test organisms to each aquarium. Sample sizes differed for each

species. Twenty grass shrimp were used in each aquarium, giving a total of 40 individuals per concentration. Ten hermit crabs were used in each aquarium, giving a total of 20 individuals per concentration. In the bay scallop bioassay, 10 and 20 individuals were used, respectively in the two aquaria at each concentration. The biomass per litre of seawater for each bioassay was 0·075 g shrimp, 0·15 g hermit crab and 0·15 g and 0·30 g scallop wet weight.

Since flowing, ambient unfiltered seawater was used, temperature, salinity and oxygen were recorded daily. Each aquarium was checked and cleaned daily and dead individuals were removed. The shrimp and crabs were fed daily with chopped clam for a two-hour period, after which all remaining food was removed. Scallops received only the food contained in the unfiltered flowing seawater. Byssal attachments of scallops were noted daily. Shell growth of scallops was noted by measuring longest rib lengths both before and after the experiment. At the conclusion of each experiment, individual animals were prepared and analysed individually for tissue concentrations of cadmium. Whole shrimp were analysed, while just the soft tissues of scallops were used. Crabs were not analysed.

Tissue samples were prepared for Cd analysis by ashing at 425 °C overnight, wet digesting in one millilitre of concentrated nitric acid per sample, then ashing again at 425 °C overnight. Samples were dissolved in 5 % nitric acid, filtered and brought up to a volume of 25 ml. A Perkin-Elmer model 303 atomic absorption instrument was used according to standard procedures (Perkin-Elmer Corporation Inc., 1966).

The mortality data from the bioassays were plotted on log-probit paper and LC_{50} values were estimated for different time periods according to recommended standard procedures (American Public Health Association, 1975). Incipient LC_{50} values for each species were estimated graphically. The data on scallop growth were analysed by one-way analysis of variance, with individual comparisons made by partitioning the sum of squares (Snedecor & Cochran, 1967). Linear regressions were fitted by the least-squares method (Snedecor & Cochran, 1967). All reported differences are significant at $\alpha = 0{\cdot}05$ unless otherwise noted.

RESULTS

Because the bioassays were conducted at ambient seawater conditions for periods up to two months, temperature, oxygen and salinity varied continuously during the course of each experiment (Table 1). Cadmium concentrations measured regularly throughout each experiment are presented in Table 2. Cumulative mortality curves for each species are presented in Fig. 1. Clearly, the response of each species to Cd differs. Hermit crabs died at all five Cd concentrations whereas grass shrimp and bay scallops died only at the three higher concentrations. Mortality of shrimp and scallops at the two lower Cd concentrations did not differ from their control mortality of 3 %. None of the control crabs died.

TABLE 1
RANGES OF AMBIENT SEAWATER CONDITIONS MEASURED IN TEST AQUARIA DURING CONTINUOUS FLOW BIOASSAYS OF CADMIUM VERSUS THREE ESTUARINE INVERTEBRATE SPECIES

	Temperature (°C)	*Salinity* (‰)	*Oxygen* (*mg/litre*)	*Dates*
P. longicarpus hermit crab	2·5–7·0	27·0–31·5	10·2–12·2	22 January–2 April, 1973
P. pugio grass shrimp	6·5–14·0	27·0–31·5	9·8–10·7	8 November–21 December, 1972
A. irradians bay scallop	9·5–16·0	27·4–31·5	8·8–10·0	23 April–4 January, 1973

Acute and chronic responses of each species to Cd may be compared. A measure of acute toxicity may be gained by considering time to 50% mortality at the highest Cd concentration, 1·0 mg/litre. For grass shrimp and hermit crabs this time period was 23 and 21 days, respectively. For bay scallops this time period was 10 days (Fig. 1). Thus, the acute response of bay scallops to Cd took place in about half the time it took for either crustacean species. A different picture emerges if we consider the responses elicited during the entire course of each bioassay. The toxicity curve for each species is plotted in Fig. 2. These curves may be used to visually estimate incipient LC_{50} concentrations, those levels at which the toxicity curve stabilises—i.e. becomes parallel to the time axis with no further deaths recorded. Bay scallop and hermit crab curves closely approach a parallel to the time axis and, therefore, incipient LC_{50} concentrations of 0·53 and 0·07 mg/litre, respectively may be estimated with some confidence. Because the grass shrimp curve did not stabilise, an incipient LC_{50} could not be estimated with confidence; however, it clearly fell well below the 0·53 mg/litre level for bay scallops and probably within the range of 0·2 to 0·3 mg/litre. Two observations are supported by the information in Fig. 2: first, while the initial acute responses of the crab and shrimp were similar (21 and 23 days, respectively to 50% mortality at 1 mg/litre Cd), their long-term responses diverged and the crabs proved to be much more sensitive to Cd; secondly, the scallops were much more sensitive than either crabs or shrimp to the initial Cd insult but the long-term responses reversed this. Both shrimp and crabs were more sensitive than scallops to long-term exposure to Cd, crabs being the most sensitive.

TABLE 2
MEAN (STANDARD DEVIATION) CADMIUM CONCENTRATIONS MEASURED IN TEST AQUARIA DURING CONTINUOUS FLOW BIOASSAYS OF THREE ESTUARINE INVERTEBRATE SPECIES

	Controls	0·06	0·12	*Nominal concentrations* (*mg/litre*)		
				0·25	0·50	1·0
P. longicarpus	0·002	0·08 (0·02)	0·14 (0·03)	0·31 (0·05)	0·62 (0·12)	1·12 (0·15)
P. pugio	—	0·07 (0·01)	0·12 (0·01)	0·27 (0·03)	0·56 (0·03)	1·10 (0·05)
A. irradians	0·003	0·07 (0·01)	0·13 (0·02)	0·26 (0·03)	0·56 (0·06)	1·14 (0·06)

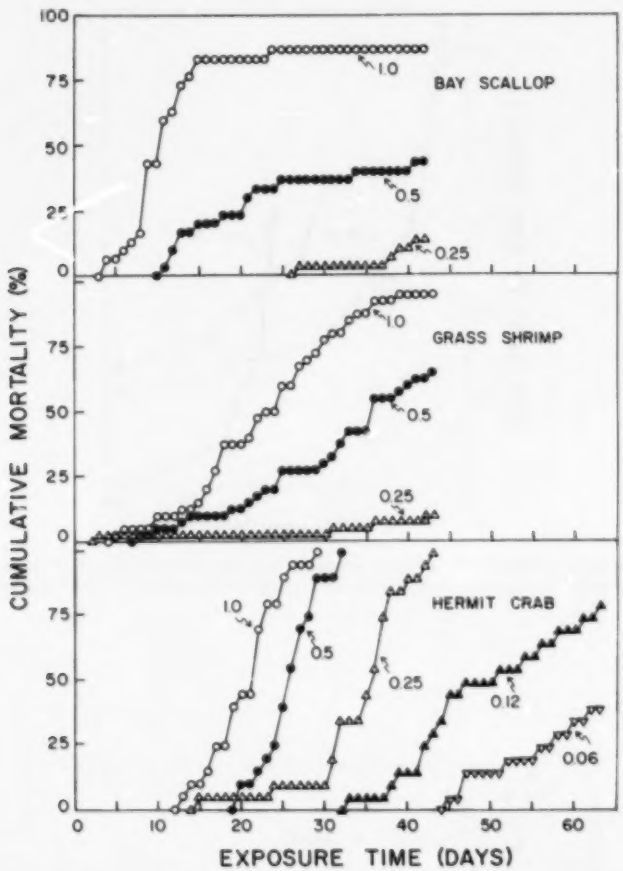

Fig. 1. Cumulative mortality expressed as percentage over time for three estuarine invertebrates exposed to five concentrations of cadmium (mg/litre) in flowing, unfiltered seawater. Control mortality was 3% for scallops, 3% for shrimp and 0% for crabs. Mortality at the 0·12 and 0·06 mg/litre Cd concentration levels did not differ from control mortality for scallops and shrimp.

Non-lethal responses of byssal attachment and growth were observed for the scallops. Juvenile bay scallops attach to firm substrates by byssal threads. We observed that prior to death the scallops would detach and lie free on the bottom of the aquaria. This detachment response took place only at Cd concentrations which eventually killed some scallops. In addition to controls, scallops in the two lowest Cd concentrations remained alive and attached throughout the bioassay. By the last

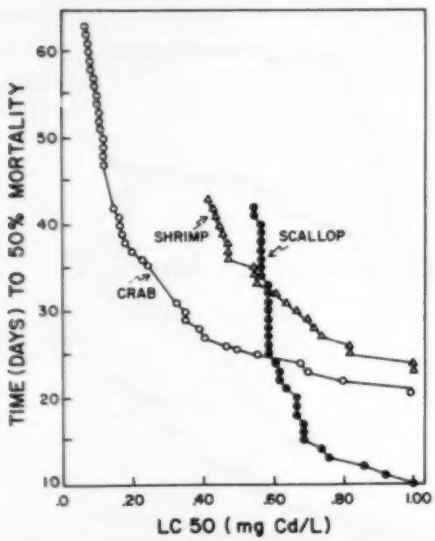

Fig. 2. Cadmium toxicity curves for three species of estuarine invertebrates. Curves represent time in days to 50% mortality for concentrations of cadmium (mg/litre) in flowing seawater.

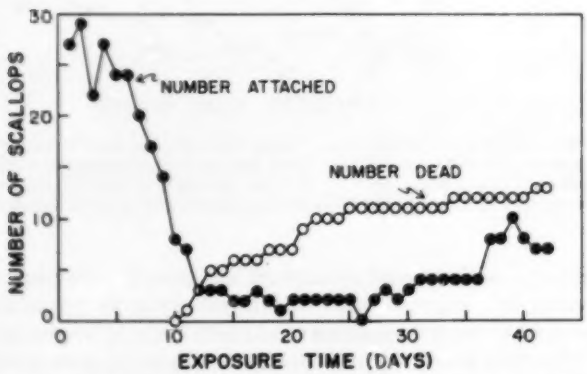

Fig. 3. Bay scallop byssal thread attachment versus mortality over time at a cadmium concentration of 0·5 mg/litre in flowing seawater.

day of the bioassay 26 of 30 scallops in the 1 mg/litre concentration had died and three of the four remaining were detached. At 0·5 mg/litre Cd 13 had died and 10 of the remaining 17 were detached, whereas at 0·25 mg/litre Cd 4 had died and 4 of the remaining 26 were detached.

The pattern of byssal attachment over time relative to the pattern of mortality over time is illustrated in Fig. 3 for scallops exposed to 0·5 mg/litre Cd. The scallops began to detach on day 4 of the bioassay and most were on the bottom of the aquaria by day 12. On day 11 mortalities were observed. Scallops continued dying until day 25, at which time the mortality curve stabilised with just two additional scallops dying by day 42. On day 29 the remaining detached scallops began to reattach. These response patterns indicate an initial traumatisation followed by many deaths. The survivors adjust to the presence of Cd, as indicated by the pattern of reattachment.

Inhibition of scallop growth by Cd is revealed by measurement of longest rib length (Table 3). Even at the lowest exposure concentration, 0·06 mg/litre Cd, there

TABLE 3

GROWTH RESPONSE OF BAY SCALLOPS EXPOSED TO CADMIUM IN FLOWING SEAWATER FOR SIX WEEKS AS INDICATED BY DIFFERENCES IN MEAN LENGTH IN MILLIMETRES OF LONGEST RIB

Cd in seawater (mg/litre)	*Before exposure*		*After exposure*		*Difference Before and After (mm)*	*Difference from control (mm)*
	N	*Mean ± SD (mm)*	*N*	*Mean ± SD (mm)*		
Control	30	13·8 ± 1·4	29	22·3 ± 2·0	8·5**	—
0·06	30	13·8 ± 1·2	30	18·7 ± 2·0	4·9**	−3·6**
0·12	30	14·0 ± 1·5	29	16·6 ± 1·9	2·6**	−5·9**
0·25	30	13·6 ± 1·4	26	13·8 ± 1·1	0·2	−8·3**
0·5	30	13·6 ± 1·7	17	12·8 ± 1·5	−0·8	−9·3**
1·0	30	13·5 ± 1·8	4	11·3 ± 0·05	−2·2**	−10·7**

**$\alpha < 0{\cdot}01$.

was a highly significant ($\alpha < 0{\cdot}01$) reduction in growth compared with controls. At 0·06 mg/litre Cd this reduction in growth was 42% compared with controls; at 0·12 mg/litre Cd the reduction was 69%. At 0·25 mg/litre Cd there was no growth. At the two highest concentrations, 0·5 and 1·0 mg/litre Cd, there was a reduction in mean size of scallops. This reduction was highly significant ($\alpha < 0{\cdot}01$) at the 1·0 mg/litre Cd level. The only plausible explanation is that larger scallops were selectively killed by the Cd, thus reducing the mean size of the survivors.

Tissue concentrations of cadmium in shrimp and scallops increased with increasing concentrations of Cd in seawater (Table 4). The increase in tissue concentrations for both species was significant ($\alpha < 0{\cdot}05$) even at the lowest exposure concentration, 0·06 mg/litre Cd. If bioconcentration factors (BCF) are considered, we find that they are inversely related to seawater concentration of Cd.

TABLE 4

MEAN (RANGE) TISSUE CONCENTRATIONS OF CADMIUM (MG/KG DRY WEIGHT) IN TWO ESTUARINE INVERTEBRATE SPECIES MEASURED AFTER EXPOSURE FOR SIX WEEKS TO ELEVATED CADMIUM CONCENTRATIONS IN FLOWING SEAWATER

Cd in seawater (mg/litre)	*Scallop mean (range)*	*BCF*[a]	*Shrimp mean (range)*	*BCF*
Controls	7·5 (7·3–7·6)		3·3 (1·4–6·2)	
0·06	1225 (1206–1253)	20400	13·4 (7·6–20·4)	223
0·12	1745 (1663–1838)	14540	20·7 (13·3–31·0)	172
0·25	2213 (2140–2346)	8850	45·0 (29·1–58·2)	180
0·5	3065 (2982–3147)	6130	52·6 (40·05–63·9)	105
1·0	—	—	106·0 (—)	106

[a] Bioconcentration Factor (BCF) = ratio of tissue concentrations: concentration in seawater.

The lowest exposure concentration, 0·06 mg/litre Cd, had the highest BCF (Table 4). The BCF values for the scallops are positively correlated with growth, $R = 0{\cdot}99$. At the lower Cd concentrations, the scallops grew and accumulated Cd from the seawater more effectively. If the Cd in scallop tissue is expressed on an amount per scallop basis, we find that the scallops from the lower exposure concentrations contain more Cd. Scallops exposed to 0·06, 0·12, 0·25 and 0·50 mg/litre Cd contained an average of 0·11, 0·11, 0·06 and 0·05 mg Cd per scallop, respectively.

DISCUSSION

There are marked species differences in sensitivity to Cd. Eisler (1971) reports 96-h LC_{50} values ranging from 0·32 to 55 ppm Cd for a variety of marine species. He found crustaceans to be most sensitive and teleosts to be least sensitive to Cd insults. Literature reports on 96-h LC_{50} values of Cd range from 114 mg/litre for the teleost *Fundulus heteroclitus* (Voyer, 1975) to 0·0155 mg/litre for the crustacean *Mysidopsis bahia* (Nimmo *et al.*, 1977*a*). We found differences among the three species we studied. The hermit crab was most sensitive to prolonged Cd exposure, being more sensitive than grass shrimp and bay scallop.

Short-term response (acute) of a particular species to Cd does not necessarily predict its long-term response (incipient LC_{50}). Eisler (1971) states that '96 h is not of sufficient duration to evaluate the toxicity of cadmium to aquatic species'. He suggests that longer exposure times are needed to properly evaluate Cd toxicity. In our study, the estimated incipient LC_{50} values ranked hermit crab, grass shrimp and bay scallop in order of increasing sensitivity. However, if acute sensitivity to Cd is judged by time to 50% mortality at the highest concentration, a different rank order emerges. Initially, the bay scallop is most sensitive and the grass shrimp is least sensitive. So not only are there marked species differences in sensitivity to Cd, but the

pattern of response over time is also different for each species. Some measure of this difference in response pattern may be gained by comparing ratios of long-term response levels (incipient LC_{50} values) with short-term levels (96-h LC_{50} values) (Table 5). These ratios range from 0·16 for the grass shrimp, *P. vulgaris* to 0·49 for the oppossum shrimp, *M. bahia*. While the oppossum shrimp is most sensitive, it responds quickly and the incipient LC_{50} level is just half the 96-h LC_{50} level. The grass shrimp is not as sensitive, nor does it respond as quickly; thus, the incipient

TABLE 5
RATIOS OF INCIPIENT LC_{50} VALUES VERSUS 96-h LC_{50} VALUES OF FIVE MARINE INVERTEBRATE SPECIES EXPOSED TO CADMIUM IN SEAWATER

Species	*Incipient* LC_{50}	96-*h* LC_{50}	*Ratio*[a]
P. vulgaris	0·12[1]	0·76[1]	0·16
P. duorarum	0·72[1]	3·50[1]	0·21
P. longicarpus	0·07	0·32[2]	0·22
M. bahia	0·0076[3]	0·0155[3]	0·49
A. irradians	0·53	1·48[4]	0·36

[a] Ratio = incipient LC_{50}· 96 h LC_{50}.
[1] Nimmo *et al.* (1977*b*).
[2] Eisler (1971).
[3] Nimmo *et al.* (1977*a*).
[4] Nelson *et al.* (1976).

LC_{50} level is approximately one-seventh the 96-h LC_{50} level. We would expect the observed Cd toxicity levels, including those in the present study, to be lowered by studies which include entire life cycles. Such studies would reveal the cumulative effects of Cd as well as the most sensitive life stages of particular species.

The toxicity of Cd depends on the free ionic form rather than total Cd. Sunda *et al.* (1978) conducted a series of experients with seawater of different salinities and different concentrations of a chelator to determine the relationship between the chemical form of Cd and the toxicity of Cd to grass shrimp, *P. pugio*. Mortality was related to the free Cd ion concentration. This concentration was determined by the total Cd concentration and the degree of complexation. The 96-h LC_{50} value for the free Cd ion was approximately 0·045 mg/litre. We found the incipient LC_{50} value of the free Cd ion to be between 0·006 and 0·012 mg/litre for grass shrimp, *P. pugio* and between 0·002 and 0·003 mg/litre for hermit crabs, *P. longicarpus*. These estimates of free ion concentration are based on information presented by Sunda *et al.* (1978). Similar studies have demonstrated that the toxicity of copper is due to its free ionic form (Davey *et al.*, 1973; Morris & Russell, 1973; Stephenson & Taylor, 1975). Complexation of either copper or cadmium reduces toxicity. To predict toxic effects of metals in marine environments it is necessary to understand the chemistry of each metal and its mode of toxic action. It is not a simple subject and deserves further study.

Non-lethal responses of growth and byssal attachment were noted for the bay scallop in our study. Growth of scallops was inhibited at all Cd concentrations studied. A calculated 42-day EC_{50} indicated that a Cd concentration of 0·078 mg/litre would reduce scallop growth to 50% of growth in controls. Cadmium concentrations up to 0·078 mg/litre have been reported for seawater sampled in Corpus Christi Bay (Holmes *et al.*, 1974). The marked inhibition of growth at all Cd concentrations studied indicates that Cd concentrations found in contaminated marine environments may be toxic to bay scallops.

The byssal thread detachment response may be a useful predictor of mortality in bay scallops. Scallops detached only in Cd concentrations which killed some of them. By the eighth day of the bioassay only five scallops had died at the highest Cd concentration. No mortality had occurred at other concentrations. However, many scallops were detached in the higher Cd concentrations. The EC_{50} for byssal attachment on day 8 was 0·54 mg/litre Cd. This value compares favourably with the estimated incipient LC_{50} value of 0·53 mg/litre Cd. Byssal thread detachment is a short-term response worthy of further study.

Cadmium is readily accumulated by marine organisms, a phenomenon which has been demonstrated for plankton (Martin & Broenkow, 1975), fish (Eisler *et al.*, 1972; Lowman *et al.*, 1971; Peden *et al.*, 1973), crustaceans (Eisler *et al.*, 1972; Benayoun *et al.*, 1974; O'Hara, 1973) and molluscs (Pringle *et al.*, 1968; Brooks & Rumsby, 1965).

At low Cd concentrations where organisms are affected less by its toxic properties, Cd is accumulated more efficiently, as measured by bioconcentration factors (BCF). Eisler (1974) reported this for Cd accumulation by mummichogs, *F. heteroclitus*. Nimmo *et al.* (1977*b*) observed the same trend in Cd accumulation studies with pink shrimp, *P. duorarum*. We also found this pattern with scallops and shrimp. The high correlation between growth and BCF exhibited by bay scallops may provide a tentative explanation for this pattern of bioaccumulation. Clearly, Cd toxicity limits growth in scallops. Where growth was limited the least, Cd accumulated to the greatest extent. If Cd accumulations were metabolically mediated, we would expect to find this pattern. In support of this hypothesis, Zaroogian & Cheer (1976) observed that during the summer, when warmer seawater temperatures support a higher metabolic rate, oysters, *Crassostrea virginica*, more than doubled in accumulation rate of Cd compared with winter conditions.

Zaroogian & Cheer (1976) measured significant levels of Cd accumulation in soft tissues of oysters, *C. virginica*, exposed to flowing water Cd levels of 0·005 mg/litre. After 40 weeks' exposure Cd concentrations reached the human emetic threshold of 13 to 15 mg/kg Cd wet weight. Eisler *et al.* (1972) exposed oysters (*C. virginica*), bay scallops (*A. irradians*), lobster (*Homarus americanus*) and mummichogs (*F. heteroclitus*) to 0·010 mg/litre Cd in flowing seawater for three weeks. Cadmium levels in treated organisms were consistently higher than those in controls. Scallops had more than doubled their tissue levels of Cd while the oysters increased by a

factor of 4·5. Nimmo *et al.* (1977*b*) exposed pink shrimp, *Penaeus dourarum*, to flowing seawater Cd concentrations ranging from 0·002 to 0·030 mg/litre for up to 50 days. The shrimp accumulated Cd in direct proportion to the concentration in the water. These three studies are significant because their Cd concentrations are in a range likely to be encountered in impacted marine environments (Holmes *et al.*, 1974).

In summary, Cd concentrations similar to those measured in impacted estuaries are lethal to oppossum shrimp and hermit crabs in controlled bioassays. These Cd concentrations suppress growth in bay scallops—an indication that Cd is deleterious to this species. Further, all marine species tested, including several of commercial importance, readily accumulate Cd. It seems prudent, therefore, to conclude that Cd poses a threat to some coastal marine species and to humans via seafood.

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